

COMPARATIVE STUDY ON LIPID PEROXIDATION AND ANTIOXIDANT VITAMINS E AND C IN *FALCIPARUM* AND *VIVAX* MALARIA

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ABSTRACT

Reactive oxygen species are thought to be involved in the pathogenesis of Malaria. To assess the extent of oxidative stress, a study was conducted in patients with *Plasmodium falciparum* malaria and *Plasmodium Vivax* malaria. Plasma Thiobarbituric acid reactive substances (TBARS) were measured to assess the degree of lipid peroxidation. Antioxidant status was measured by estimating the levels of Vitamins E and C. Results were compared with age and sex matched control subjects. This study suggests that plasma TBARS levels were significantly increased in malaria patients. The patients with *P. falciparum* infection showed significantly increased levels of lipid peroxides when compared to *P. vivax* malaria. The antioxidant Vitamins E and C were decreased significantly in malaria patients in both the groups. Maximum decline in Vitamin C was observed in *P. vivax* malaria. Therefore it is been hypothesized that antioxidant Vitamins E and C could provide protection against the oxidative stress induced by malaria.

KEY WORDS

Malaria, Lipid peroxidation, Antioxidant Vitamins E & C.

INTRODUCTION

Malaria is a major health problem in developing countries accounting for 2-3 million deaths per year (1,2). Malaria is caused in man by protozoan parasite of the genus *Plasmodium* and transmitted by the infected mosquitoes. The life cycle of the plasmodium species is completed in two hosts. The primary host being the anopheline mosquito and the secondary host being the human beings. Sporozoites inoculated with the saliva of an infected female mosquito into a susceptible host, reach the blood stream and later invades hepatocytes, where they multiply to produce merozoites. These merozoites invade red cells and undergo further cycle of asexual reproduction, called erythrocyte schizogony (3). During its entry and subsequent growth, the parasite produces distinct structural and functional changes in the host erythrocyte membrane. These changes become prominent as the parasite develops from the ring to the schizont stage inside the cells (4,5).

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Oxidative stress plays an important role in the development of malarial anemia (6,7). Malarial infection activates the immune system of the body thereby causing the release of reactive oxygen species (ROS). The malaria parasite itself generates large quantities of reactive oxygen species and also through its interaction with phagocytes (8,9,10).

Some of these radicals traverse the membrane by anionic channel attacking the plasma membranes and intracellular hemoglobin (11,12,13). Oxidative stress is also aggravated by decreased antioxidant defence system. Parasite utilizes erythrocyte proteins for its metabolic requirements, the concentration of enzymic antioxidants are decreased with parasite maturation (14).

Reports on ROS production in human *P. falciparum* infection are few. The role of antioxidants and oxidative stress in the pathogenesis of malaria in humans is unclear. The present study was therefore undertaken to determine the extent of lipid peroxidation and to investigate the alterations in major antioxidant Vitamins E and C in *P. falciparum* malaria and *P. vivax* malaria.

MATERIALS AND METHODS

The study population consisted of 28 untreated malaria patients between the age group 16-52 years of both sexes. These patients attended OPD at Wenlock District Hospital, Mangalore with the symptoms of fever and rigor, headache, vomiting, signs include splenomegaly, hepatomegaly and anemia. The control group included 26 healthy individuals of both sexes between 20 to 55 years.

A finger prick blood sample was taken to prepare thick and thin blood films to determine the presence or absence of malaria parasites. Patients with malaria were enrolled in the study after informed consent was obtained from the patients. Of the total 28 malaria patients, 19 patients had *P. vivax* malaria and 9 patients had *P. falciparum* malaria.

SAMPLE COLLECTION : 5 ml of venous blood samples were collected randomly in EDTA bottles from malaria patients and normal healthy individuals. Blood samples were centrifuged at 3000g for 10 minutes. Plasma was collected taking care to avoid hemolysis and used for the estimation of lipid peroxidation, vitamins E and C.

1. **Lipid Peroxidation :** The plasma lipid peroxidation (Thiobarbituric acid reactive substances TBARS) was studied by the method proposed by Satoh (15).
2. **Vitamin E (alpha – Tocopherol) :** was measured using the method of Bieri et al (16).
3. **Vitamin C (Ascorbic acid) :** in plasma was measured by 2,4 – Dinitro phenyl hydrazine method (17).

Statistical Analysis : Statistical analysis was done by using Mann Whitney 'U' test. Correlations between the variables were estimated by Pearson's Correlation coefficients.

RESULTS

The increase in plasma TBARS in malaria patients is very highly significant ($p<0.001$) when compared to control subjects. This indicates that lipid peroxidation is significantly increased in malaria. The patients with *P. falciparum* infection showed significantly increased levels of lipid peroxides as compared to *P. vivax* malaria patients. The plasma Vitamin E concentration decreased significantly ($p<0.001$) in malaria patients compared to normal subjects. A decline in Vitamin E concentration was more in *P. falciparum* malaria as compared to *P. vivax* malaria.

Plasma Vitamin C concentration decreased in malaria patients compared to control subjects. Maximum decline in plasma

Table 1

Comparison of Plasma levels of TBARS, Vitamin E and Vitamin C in malaria patients and Controls (Mean \pm S D)

Groups	TBARS nmol/L	Vitamin E mg/L	Vitamin C mg/L
Control (n = 26.)	185.397 \pm 118.79	7.129 S \pm 2.890	1.779 \pm 0.403
<i>P. falciparum</i> (n = 9)	826.17 \pm 174.72	2.873 \pm 1.019	1.113 \pm 0.437
p – Value	< 0.001	< 0.001	NS
<i>P. Vivax</i> (n=13)	750.30 \pm 399.85	3.020 \pm 1.218	0.784 \pm 0.434
p – Value	< 0.001	< 0.001	< 0.001

n - Number of samples; p - Value Calculated by Mann Whitney test;
NS - Not significant

Vitamin C was observed in *P. vivax* infection ($p< 0.001$) as compared to *P. falciparum* malaria (Table 1).

A weekly positive correlation ($r = 0.09$) was obtained between TBARS and Vitamin E concentration in the study group which was statistically not significant. Among the controls a positive correlation ($r = 0.312$) of TBARS with Vitamin E concentration was seen. A negative correlation ($r = - 0.111$) was obtained between TBARS and Vitamin C concentration in the study group where as strong negative correlation ($r = - 0.287$) was obtained between TBARS and Vitamin C concentration in the control group.

DISCUSSION

The present study shows significant increase in lipid peroxides in malaria patients as compared to control subjects. The patients with *P. falciparum* infection showed highest levels of lipid peroxides as compared to *P. vivax* malaria patients.

Invasion of human erythrocytes by malaria parasite brings about metabolic changes in the host cell. The host cells may then become more vulnerable to damage due to toxic metabolites derived from both the host and parasites. Reactive oxygen species generated in host-parasite interactions causes the lysis of erythrocytes and alteration in antioxidants (18,19,20).

There are many sources of ROS in malaria such as generation by intra erythrocyte parasite, production by host-phagocytes as a defence mechanism against the parasite. ROS are also generated during the consumption of hemoglobin by the malaria parasite.

P. falciparum trophozoite infected human red cells produce more ROS compared to vivax infections and control groups as evident from increased TBARS levels in the plasma in our studies.

Beside this the plasma of malaria patients has been suspected to contain pro-oxidants. Hemoglobin can act as pro-oxidant by catalyzing the decomposition of lipid hydroperoxides by enhancing the chain reaction (21). H₂O₂ are capable of accelerating lipid peroxidation. Excess H₂O₂ could also results in breakdown of heme and release of free iron ions, which in turn form OH· (22). ROS can cause damage to malaria parasite as well as to non parasitized erythrocytes.

All these factors cause a substantial rise in lipid peroxidation which may lead on to Oxidative stress in malaria.

The present study showed significant reduction in plasma Vitamins E & C concentration in malaria patients as compared to controls. The decline in Vitamin E was more in *P. falciparum* malaria as compared to *P. vivax* malaria where as maximum decline in Vitamin C was observed in *P. vivax* malaria.

The decrease in antioxidant Vitamins E & C in the patient groups might be due to their transfer to red blood cell membrane to counteract the increase oxidative stress during acute phase of disease by inhibiting membrane lipid peroxidation or due to their increased utilization as plasma antioxidants. The impaired release of antioxidant Vitamin E may also occur during acute phase of disease (23). Vitamin E accounts for most of the chain breaking antioxidant activity in the erythrocyte membrane. To be functionally active, tocopherol has to be maintained in its native state for which Ascorbate contributes. Ascorbic acid (Vitamin C), converts tocopheroxyl radical to its native state. Therefore loss of ascorbate may interfere in Tocopherol regeneration and may lead to impaired membrane function.

In the present study, the plasma Vitamin E was weakly positive correlated with TBARS. Whereas plasma Vitamin C was negatively correlated with TBARS concentration. Besides this the reduced antioxidant vitamin E and C content of plasma also augmented lipid peroxidation. Reduced levels of plasma ascorbate and Tocopherol observed in the present study may have contributed to oxidative destruction of erythrocytes.

From these studies it can be concluded that the substantial increase in lipid peroxides in malaria patients might be the result of reactive oxygen species production, by the activated immune system, by the parasite itself and blood phagocytes.

Administration of antioxidant Vitamins E and C along with the treatment for parasite clearance may be fruitful to avoid malarial anemia and excessive hemolysis.

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